

What is claimed is:

- 1/ A method for the expression of high yields of active protein-Ig fusions comprising
culturing a host transformed with DNA encoding a desired protein-Ig fusion in a
5 culture system having a low temperature of about 27° C to about 35° C.
2. The method of claim 2 wherein the temperature is about 27°C to about 32° C.
3. The method of claim 3 wherein said transformed host is first cultured at a temperature
above about 33° C for a period of time sufficient to allow growth of said host.
4. The method of claim 1 wherein said protein-Ig fusion comprises a member of the TNF
10 receptor family.
5. The method of claim 3 wherein said TNF receptor family member is a lymphotoxin-β
receptor, TNFR-55, HVEM or a fragment thereof.
6. The method of claim 1 further comprising the step of recovering active protein-Ig
fusions from said culture system by hydrophobic interaction chromatography.
- 15 7. The method of claim 1 wherein said culture system comprises insect or bacterial cells.
- 8/ An active protein-Ig fusion obtained by culturing a host transformed with DNA
encoding the fusion in a culture system having a low temperature of about 27° C to
about 35 ° C.
9. The fusion of claim 8 comprising a member of the TNF family.
- 20 10. The fusion of claim 9 comprising LT-β receptor, or a fragment thereof.
11. The fusion of claim 9 comprising HVEM, or a fragment thereof.
- 12/ A method of making a pharmaceutical preparation comprising an active protein-Ig
fusion said method comprising:
- (a) culturing a host transformed with DNA encoding the protein-Ig fusion in a culture
25 system having a low temperature of about 27° C to about 32 ° C, thereby
expressing
active protein-Ig fusions;
- (b) recovering active protein-Ig fusions from said culture system; and
- (c) combining the active protein-Ig fusions of step (b) with a pharmaceutically
30 acceptable carrier.

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FOOTNOTES

13. The method of claim 12 wherein the protein-Ig fusion comprises a member of the TNF family, or a fragment thereof.
14. The method of claim 13 wherein the protein-Ig fusion comprises lymphotoxin- β receptor or a fragment thereof.
- 5 15. The method of claim 13 wherein the protein-Ig fusion comprises HVEM, or a fragment thereof.
16. A pharmaceutical preparation obtained by
- 10 (a) culturing a host transformed with DNA encoding the protein-Ig fusion in a culture system having a low temperature of about 27° C to about 32 ° C, thereby expressing active protein-Ig fusions;
- (b) recovering active protein-Ig fusions from said culture system; and
- (c) combining the active protein-Ig fusions of step (b) with a pharmaceutically acceptable carrier.
- 15 17. The pharmaceutical preparation of claim 16 wherein the protein-Ig fusion comprises a member of the TNF family.
18. The pharmaceutical preparation of claim 17 wherein the protein-Ig fusion comprises a lymphotoxin- β receptor or a fragment thereof.
19. The pharmaceutical preparation of claim 17 wherein the protein-Ig fusion comprises HVEM, or a fragment thereof.
- 20 20. A method for the expression of high yields of active protein-Ig fusions comprising culturing yeast transformed with DNA encoding a desired protein-Ig fusion in a culture system having a low temperature of about 10° C to about 25° C.
21. The method of claim 20 wherein the temperature is about 15°C to about 20° C.
22. The method of claim 20 wherein said transformed host is first cultured at a temperature
- 25 above about 30° C for a period of time sufficient to allow growth of said host.
23. The method of claim 20 wherein said protein-Ig fusion comprises a member of the TNF receptor family.
24. The method of claim 23 wherein said TNF receptor family member is a lymphotoxin- β receptor or a fragment thereof.
- 30 25. The method of claim 20 further comprising the step of recovering active protein-Ig fusions from said culture system by hydrophobic interaction chromatography.

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26. An active protein-Ig fusion obtained by culturing yeast transformed with DNA encoding the fusion in a culture system having a low temperature of about 10° C to about 25 ° C.

27. The fusion of claim 26 comprising a member of the TNF family.

5 28. The fusion of claim 27 comprising LT- β receptor, or a fragment thereof.

29. The fusion of claim 26 comprising HVEM, or a fragment thereof.

30. A pharmaceutical preparation comprising an active protein-Ig fusion having an Ig Fc domain and peptide chains, wherein the Ig Fc domain is altered thereby altering the rate of disulfide formation in the hinge region of said protein-Ig fusion.

10 31. The preparation of claim 30, wherein said Ig Fc domain is altered by replacing at least one cysteine residue with alanine.

32. A protein-Ig fusion comprising an Ig-Fc domain crosslinked to a peptide derived from the TNF family wherein at least one cysteine residue on the Ig-Fc domain is replaced with alanine.

15 33. The protein-Ig fusion of claim 32 wherein said peptide is derived from a lymphotoxin- β receptor.

34. A method of making a protein-Ig fusion comprising an Ig Fc domain crosslinked to a peptide derived from the TNF receptor family by mutagenizing at least one cysteine residue to an alanine, thereby increasing the yield of active forms of fusion expressed.

20 35. The method of claim 34 wherein said peptide is LTBR, and the cysteines at positions 101 and 108 are mutagenized to alanines.

36. An LTBR-Ig fusion protein comprising alanine at positions 101 and 108 of the LTBR peptide.

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